

CLAIMS

1. A decellularized tissue which has been subjected to a radical reaction.
2. A decellularized tissue according to Claim 1, characterized in that said decellularized tissue is substantially free of solubilized protein.
3. A decellularized tissue according to Claim 1, characterized in that a extracellular matrix component is at least partially crosslinked by means of covalent bonding.
4. A decellularized tissue according to Claim 3, wherein the extracellular matrix component is selected from the group consisting of collagen, elastin, laminin, fibronectin, glycosaminoglycan and proteoglycan.
5. A decellularized tissue according to Claim 3, wherein the crosslinking is formed between two or more amino acids having no free amino group or carboxy group at the side chain thereof.
6. A decellularized tissue according to Claim 3, wherein the crosslinking is formed on the groups other than carboxyl, hydroxyl, and amino groups.
7. A decellularized tissue according to Claim 6, wherein the group comprises a group selected from the group consisting of sulphhydryl group, aldehyde group, carbonyl group, sulfo group and nitro group, and the crosslinking is formed by

means of a bonding selected from the group consisting of carbon-carbon bonding, ether bondin and ester bonding.

8. A decellularized tissue according to Claim 1, wherein

- A) a cell residual rate of the tissue is less than a level at which an immune reaction is elicited in an organism; and
- B) the tissue is not damaged to such an extent that hinders the tissue from exhibiting a function which was possessed by the tissue when the tissue was not decellularized.

9. A decellularized tissue according to Claim 1, wherein the radical reaction comprises exposure to a free radical.

10. A decellularized tissue according to Claim 1, wherein the tissue strength thereof is substantially the same as that of a decellularized tissue without exposure to a radical reaction, whereas decellularization thereof is significantly progressed.

11. A decellularized tissue according to Claim 1, wherein the radical reaction comprises a treatment selected from the group consisting of gamma-ray irradiation, ultraviolet irradiation, exposure to a free radical source, exposure to ultrasonication, electron beam irradiation, and x-ray irradiation.

12. A decellularized tissue according to Claim 1, wherein the radical reaction is gamma-ray irradiation, and the dose of the gamma-ray irradiation is between 10 and 250 kGy.

13. A decellularized tissue according to Claim 1, wherein the radical reaction is gamma-ray irradiation conducted under an atmosphere selected from the group consisting of in vacuum, in oxygen, in nitrogen, in the atmosphere, in water, in an amphipathic molecule solution and a combination thereof.
14. A decellularized tissue according to Claim 1, wherein the decellularized tissue is subjected to DNase treatment.
15. Decellularized tissue according to Claim 1, wherein the tissue is treated in a solution containing a non-micellar amphipathic molecule.
16. Decellularized tissue according to Claim 15, wherein the solution containing the non-micellar amphipathic molecule comprises a 1,2-epoxide polymer.
17. Decellularized tissue according to Claim 15, wherein the solution containing the non-micellar amphipathic molecule comprises polyethylene glycol (PEG).
18. Decellularized tissue according to Claim 17, wherein an average molecular weight of the PEG is between 200 to 6000.
19. Decellularized tissue according to Claim 17, wherein an average molecular weight of the PEG is about 1000.

20. Decellularized tissue according to Claim 1, wherein the cell residual rate of the tissue is about 5 % or less.
21. Decellularized tissue according to Claim 1, wherein the cell residual rate of the tissue is about 4 % or less.
22. Decellularized tissue according to Claim 1, wherein the cell residual rate of the tissue is about 1 % or less.
23. Decellularized tissue according to Claim 1, wherein the tissue has substantially no cells remaining.
24. Decellularized tissue according to claim 1, wherein the tissue damage rate of the tissue is about 30% or less.
25. Decellularized tissue according to claim 1, wherein the tissue damage rate of the tissue is about 15% or less.
26. Decellularized tissue according to claim 1, wherein the tissue damage rate of the tissue is about 5% or less.
27. Decellularized tissue according to claim 1, wherein the tissue has a tissue strength which permits a clinical application.

28. Decellularized tissue according to claim 1, wherein the tissue has a tissue strength which is 80% or more of the tissue strength which was possessed by the tissue when the tissue was not decellularized.
29. Decellularized tissue according to claim 1, wherein the tissue has a tissue strength having a β value which is 80% or more of the β value which was possessed by the tissue when the tissue was not decellularized.
30. Decellularized tissue according to claim 1, wherein the tissue has a tissue strength having a β value of 20 or more.
31. Decellularized tissue according to claim 1, wherein the tissue is luminal tissue.
32. Decellularized tissue according to claim 1, wherein the tissue is tissue selected from blood vessels, blood vessel-like tissue, cardiac valves, pericardia, dura mater, corneas, and bones.
33. Decellularized tissue according to claim 1, wherein a survival rate of naive extracellular matrix in the decellularized tissue is at least about 50%.
34. Decellularized tissue according to claim 33, wherein the survival rate of the extracellular matrix is substantially a hundred percent.

35. Decellularized tissue according to claim 1, wherein the tissue is derived from a mammal.
36. Decellularized tissue according to claim 1, wherein the tissue is derived from a human.
37. Decellularized tissue according to claim 1, wherein the tissue is derived from a swine.
38. Decellularized tissue according to claim 1, wherein viruses have been substantially removed from the tissue.
39. Decellularized tissue according to claim 38, wherein the viruses are selected from the group consisting of retroviruses and herpes viruses.
40. Decellularized tissue according to claim 38, wherein the viruses comprises porcine endogenous retrovirus (PERV).
41. Decellularized tissue according to claim 40, wherein the PERV is removed such that no significant infection to a human cell is observed.
42. A tissue graft comprising decellularized tissue according to Claim 1.
43. A tissue graft according to Claim 42 further comprising a cell.

44. A tissue graft according to Claim 42, wherein the tissue graft has a form selected from the group consisting of membrane-form, luminal form and valvular form.

45. A method of producing decellularized tissue, comprising the steps of:

- 1) providing tissue;
- 2) immersing the tissue in a solution containing a non-micellar amphipathic molecule; and
- 3) subjecting the tissue to a radical reaction.

46. A method according to Claim 45, wherein the radical reaction comprises exposure to a free radical.

47. A method according to Claim 45, wherein the tissue strength thereof is substantially the same as that of a decellularized tissue without exposure to a radical reaction, whereas decellularization thereof is significantly progressed.

48. A method according to Claim 45, wherein the radical reaction comprises a treatment selected from the group consisting of gamma-ray irradiation, ultraviolet irradiation, exposure to a free radical source, exposure to ultrasonication, electron beam irradiation, and x-ray irradiation.

49. A method according to Claim 45, wherein the radical reaction is gamma-ray irradiation, and the dose of the gamma-ray irradiation is between 10 and 250 kGy.

50. A method according to Claim 45, wherein the radical reaction is gamma-ray irradiation, and the dose of the gamma-ray irradiation is between 40 and 100 kGy.
51. A method according to Claim 45, wherein the radical reaction is gamma-ray irradiation conducted under an atmosphere selected from the group consisting of in vacuo, in oxygen, in nitrogen, in the atmosphere, in water, in an amphipathic molecule solution and a combination thereof.
52. A method according to Claim 45, wherein the radical reaction is conducted in the atmosphere.
53. A method according to Claim 45, wherein the tissue is treated in a solution containing a non-micellar amphipathic molecule.
54. A method according to Claim 53, wherein the solution containing the non-micellar amphipathic molecule comprises a 1,2-epoxide polymer.
55. A method according to Claim 53, wherein the solution containing the non-micellar amphipathic molecule comprises polyethylene glycol (PEG).
56. A method according to Claim 55, wherein the average molecular weight of the PEG is between about 200 to about 2000.
57. A method according to Claim 55, wherein the average molecular weight of the PEG is between about 1000 to about 2000.

58. A method according to Claim 55, wherein the PEG solution is a solution of about 60% to about 100%.

59. A method according to Claim 45, wherein the tissue is a luminal tissue, a valvular tissue or a membranous tissue.

60. A method according to Claim 45, wherein the tissue is tissue selected from blood vessels, blood vessel-like tissue, cardiac valves, pericardia, dura mater, corneas, and bones.

61. A method according to Claim 45, wherein the tissue is derived from a mammal.

62. A method according to Claim 45, wherein the tissue is derived from a human or a swine.

63. A method according to Claim 45, wherein the step of immersing is conducted for 30 minutes to ten days.

64. A method according to Claim 45, wherein the step of immersing further comprises a step of physical treatment.

65. A method according to Claim 45, further comprising the step of D) washing the tissue.

66. A method according to Claim 65, wherein the step of washing is conducted for half a day to five days.

67. A method according to Claim 45, wherein the amphipathic molecule is biocompatible.

68. A method according to Claim 45, wherein the step of immersing comprises a step of agitation.

69. A method according to Claim 45, wherein the step of immersing comprises agitation for one week or longer.

70. A method according to Claim 45, wherein the decellularized tissue is subjected to a DNase treatment.

71. A method according to claim 45, further comprising:
4) performing chemical treatment.

72. A method according to claim 68, wherein the chemical treatment is performed with DNase.

73. A method according to claim 68, wherein the chemical treatment is performed with DNasel.

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74. A method according to Claim 72, wherein the treatment by the DNase is conducted for 24 hours or longer.

75. A method according to Claim 45, wherein the radical reaction is gamma-ray irradiation and the period of irirradiation of the gamma-ray irradiation is a period so as to total a dose of about 10-300kGy.

76. A method according to claim 45, further comprising:
disseminating a cell.

77. Decellularized tissue obtainable by a method according to Claim 45.

78. A method for tissue regeneration, comprising the steps of:
a) providing decellularized tissue subjected to a radical reaction, to an organism; and
b) incubating the tissue within the organism for a time sufficient for the tissue to regenerate.

79. A method according to Claim 78, wherein the radical reaction comprises exposure to a free radical.

80. A method according to Claim 78 further comprising the step of providing a cell to the decellularized tissue.

81. A method according to Claim 80, wherein the cell is derived from the organism.
82. A method according to Claim 80, wherein the cell is present within the organism.
83. A method according to Claim 80, wherein the cell is derived from a host homologous to the organism.
84. A method according to Claim 80, wherein the cell is derived from a host heterologous to the organism.
85. A method according to Claim 80, wherein the cell is previously isolated from the organism.
86. A method according to Claim 80, the cell is a blood vessel cell or a blood vessel-like cell.
87. A method according to Claim 78, further comprising providing a physiologically active substance capable of inducing cell differentiation to the organism.
88. A method according to Claim 87, wherein the physiologically active substance is derived from or foreign to the organism.

89. A method according to Claim 87, wherein the physiologically active substance is provided in a form of a nucleic acid or a polypeptide.

90. A method according to Claim 87, wherein the physiologically active substance is selected from the group consisting of HGF, VEGF, FGF, IGF, PDGF, and EGF.

91. A method according to Claim 78, wherein the tissue is tissue selected from the group consisting of blood vessels, blood vessel-like tissue, cardiac valves, pericardia, dura mater, corneas, and bones.

92. A method of producing a tissue graft, comprising the steps of:

- A) providing decellularized tissue subjected to a radical reaction, to an organism;
- B) allowing a self cell in the organism to infiltrate the decellularized tissue; and
- C) incubating the tissue within the organism for a time sufficient for the cell to differentiate.

93. A method according to Claim 92, wherein the radical reaction comprises exposure to a free radical.

94. A method according to Claim 92, the cell is a blood vessel cell or a blood vessel-like cell.

95. A method according to Claim 92, wherein the tissue is tissue selected from the group consisting of blood vessels, blood vessel-like tissue, cardiac valves, pericardia, dura mater, corneas, and bones.

96. A method according to Claim 92, wherein the decellularized tissue further comprises a cell.

97. A method according to Claim 92, further comprising the step of D) providing a physiologically active substance capable of inducing differentiation of the cell.

98. A method according to Claim 97, wherein the physiologically active substance is a cytokine having hematopoiesis activity.

99. A tissue graft, produced by a method according to claim 92.

100. A method for treating a subject requiring implantation of tissue or an organ or treating a subject at a risk of implantation of tissue or an organ for prophylaxis, the method comprising the steps of:

A) providing decellularized tissue subjected to a radical reaction, or a tissue graft comprising the decellularized tissue; and

B) implanting the decellularized tissue or tissue graft to the subject.

101. A method according to Claim 100, wherein the radical reaction comprises exposure to a free radical.

102. A method according to Claim 100, the tissue further comprises a cell.

103. A method according to Claim 100, wherein the tissue has no cells.

104. A method according to Claim 100, wherein the tissue is derived from the subject.

105. A method according to Claim 100, wherein the tissue is tissue selected from blood vessels, blood vessel-like tissue, cardiac valves, pericardia, dura mater, corneas, and bones.

106. A method according to Claim 100, wherein the tissue is derived from a mammal.

107. A method according to Claim 100, wherein the tissue is derived from a human.

108. A pharmaceutical for organ transplantation comprising:

A) decellularized tissue subjected to a radical reaction, or a tissue graft comprising the decellularized tissue.

109. A pharmaceutical according to Claim 108, wherein the radical reaction comprises exposure to a free radical.

110. A pharmaceutical according to Claim 108, wherein the tissue is tissue selected from blood vessels, blood vessel-like tissue, cardiac valves, pericardia, dura mater, corneas, and bones.

111. A pharmaceutical according to Claim 108, wherein the tissue is derived from a mammal.

112. A pharmaceutical according to Claim 108, wherein the tissue is derived from a human or a swine.

113. A pharmaceutical according to Claim 108, wherein the tissue is derived from a subject in need of the transplantation.

114. Use of decellularized tissue subjected to a radical reaction, or a tissue graft comprising the decellularized tissue for manufacture of a pharmaceutical for organ transplantation.

115. Use according to Claim 114, wherein the radical reaction comprises exposure to a free radical.